

In the Claims:

Please substitute pending claims 19, 21, 24, 26-28, 30, 32, 36, 39 and 41- 45 with the following claims 19, 21, 24, 26-28, 30, 32, 36, 39 and 41- 45:

19. (Once Amended) A method of determining the proportion of apoptotic cells in a population of cells which have been transfected with a plasmid containing a DNA sequence of interest, comprising:

(A) transiently transfecting a population of mammalian cells with a plasmid containing a DNA sequence of interest;

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(B) co-expressing in the population of cells a fluorescent marker protein;

(C) culturing the cells in a nutrient medium so that said DNA sequence of interest or its expressed polypeptide exerts its activity on the apoptosis of the cell;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that a measurable amount of fluorescent protein expressed remains in the cells, while apoptotic DNA fragments diffuse out;

(E) measuring the proportion of the harvested cells from (D) containing a

DNA content of less than 2N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made;

(F) also, simultaneously measuring the proportion of the harvested cells in (D) containing fluorescent marker protein and thereby determining the proportion of the harvested cells transfected with the plasmid containing a DNA sequence of interest; and

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(G) comparing the values obtained in (E) and (F);

thereby, determining the proportion of apoptotic cells in the transfected population.

21. (Once Amended) The method of claim 19, wherein the fluorescent marker protein in (B) is Green Fluorescent Protein.

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24. (Once Amended) The method of claim 19, wherein the fixing and permeabilization in (D) is achieved using paraformaldehyde and ethanol, respectively.

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26. (Once Amended) A method of determining whether a DNA sequence of interest has an effect on apoptosis of cells in a culture, comprising:

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(A) transiently transfecting a population of mammalian cells with a plasmid

containing a DNA sequence of interest, thereby obtaining population X; and transiently transfecting another population of the same cells with a control plasmid, thereby obtaining a population Y;

(B) co-expressing in the population of cells a fluorescent marker protein;

(C) culturing the cells in a nutrient medium so that said DNA sequence of interest or its expressed polypeptide exerts its potential activity on the apoptosis of the cell;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that a measurable amount of fluorescent protein expressed remains in the cells, while apoptotic DNA fragments diffuse out;

(E) measuring the proportion of the harvested cells from (D) containing a DNA content of less than $2N$ and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made;

(F) also, simultaneously measuring the proportion of the harvested cells in (D) containing fluorescent marker protein and thereby determining the proportion of the harvested cells transfected with said DNA sequence of interest; and

(G) for each of the populations X and Y, comparing the values obtained in (E) and (F), thereby calculating a proportion of apoptotic cells; and

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(H) comparing the calculated proportion of apoptotic cells obtained in (G) for the populations X and Y

thereby, determining whether said DNA sequence of interest affects the proportion of apoptotic cells in the transfected population.

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27. (Once Amended) The method of claim 26, wherein the mammalian cells in (A) are tumor cells; and wherein the sequence of interest in (A) encodes a dominant negative signal transduction molecule of a receptor for a survival factor.

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28. (Once Amended) The method of claim 26, wherein the mammalian cells in (A) are tumor cells; and wherein the sequence of interest in (A) encodes a dominant negative receptor for a survival factor.

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29. (Twice Amended) The method of claim 28, wherein the dominant negative receptor is the Fibroblast Growth Factor (FGF) receptor.

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32. (Once Amended) A method of determining the effect of a test substance on the pro- or anti-apoptotic activity of a DNA sequence of interest, comprising:

(A) transiently transfecting two populations of mammalian cells with an identical plasmid containing a DNA sequence of interest;

(B) co-expressing in the population of cells a fluorescent marker protein;

(C) culturing one population of transfected cells in a suitable nutrient media containing a test substance, thereby obtaining a population X; and incubating the other population of transfected cells in a medium lacking the test substance, thereby obtaining a population Y;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that a measurable amount of fluorescent protein expressed remains in the cells, while apoptotic DNA fragments diffuse out;

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(E) measuring the proportion of the harvested cells from (D) containing a DNA content of less than 2N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made;

(F) also, simultaneously measuring the proportion of the harvested cells in (D) containing fluorescent marker protein and thereby determining the proportion of the harvested cells transfected with the plasmid containing said DNA sequence of interest; and

(G) for each of the populations X and Y, comparing the values obtained in (E) and (F), thereby calculating a proportion of apoptotic cells; and

(H) comparing the calculated proportion of apoptotic cells obtained in (G)

for the populations X and Y;

thereby, determining whether the test substance has an effect on the pro- or anti-apoptotic activity of said DNA sequence of interest.

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33. (Once Amended) The method of claim 32, wherein the mammalian cells in (A) are tumor cells; the gene of interest in (A) is a dominant negative version of a signal transduction molecules of a receptor for a survival factor particular to a tumor cell, which causes the inhibition or absence of a survival factor function; and the test substance exhibits activity synergistic with the inhibition or absence of the survival factor function.

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36. (Twice Amended) The method of claim 34, wherein the receptor for a survival factor is the Fibroblast Growth Factor (FGF) receptor.

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39. (Once Amended) The method of claim 32, wherein the test substance in (C) acts synergistically with chemotherapy.

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41. (Once Amended) A kit for the simultaneous measurement of the fluorescence of marker protein and DNA content in order to determine the proportion of apoptotic cells in a culture which comprises a carrier means being compartmentalized to receive in close confinement one or more container means wherein:

(A) a first container means holds one or more components suitable for transfection;

(B) another container means holds a plasmid containing the sequence coding for a fluorescent marker protein;

(C) another container means holds an empty vector for inserting the DNA sequence of interest and for control measurements;

(D) another container means holds a primary fixing solution;

 (E) another container means holds a secondary fixing/permeabilizing solution;

(F) another container means holds washing solution(s); and

(G) a final container means holds a DNA-binding stain.

42. (Once Amended) The kit in claim 41, wherein the one or more components suitable for transfection in (A) can achieve receptor-mediated endocytosis using polyethyleneimine and psoralen/UV-inactivated Adenovirus.

43. (Once Amended) The kit in claim 41, wherein the fluorescent marker protein in (B) is Green Fluorescent Protein.

44. (Once Amended) The kit in claim 41, wherein the primary fixing solution in (D) is 2% (w/v) paraformaldehyde and the secondary fixing/permeabilization solution in (E) is 70% ethanol.

45. (Once Amended) A method of identifying a gene which modulates apoptosis comprising:

(A) transiently transfecting a cDNA expression library into a population of cells;

(B) co-expressing in said population of cells a fluorescent marker protein;

(C) culturing the cells in a nutrient medium so that a DNA sequence of interest or its expressed polypeptide exerts its activity on the apoptosis of the cell;

(D) harvesting the cells from (C) and fixing and permeabilizing the cells so that a measurable amount of fluorescent protein expressed remains in the cells, while apoptotic DNA fragments diffuse out;

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(E) measuring the proportion of the harvested cells from (D) containing a DNA content of less than 2N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made;

(F) also, simultaneously measuring the proportion of the harvested cells in (D) containing fluorescent marker protein and thereby determining the proportion of the harvested cells transfected with said DNA sequence of interest; and

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(G) using FACS to isolate cells which deviate from an apoptosis background which is to be determined; and

(H) isolating and amplifying the cDNA from said isolated cells

thereby identifying a gene which modulates apoptosis.
